Effect of long-chain fatty acids on low-density-lipoprotein-cholesterol metabolism\textsuperscript{1–4}

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ABSTRACT The concentration of cholesterol in the low-density-lipoprotein (LDL) fraction of plasma is one of the major risk factors for coronary heart disease. Steady-state concentrations of LDL cholesterol in the plasma are determined primarily by the production rate and the rate of removal of LDL cholesterol from the circulation by receptor-dependent transport. The magnitude of these two processes is affected by the type of fatty acid in the diet. Saturated fatty acids with 14 and 16 carbon atoms suppress receptor-dependent LDL-cholesterol transport into the liver, increase the LDL-cholesterol production rate, and raise the plasma LDL-cholesterol concentration. The 9-cis 18:1 fatty acid restores receptor activity, lowers the production rate, and decreases the plasma LDL-cholesterol concentration. In contrast with these fatty acids, the 18:0 and 9-trans 18:1 fatty acids are biologically inactive and so do not change the circulating LDL-cholesterol concentration. The alternative situation in which the cholesterol content of the diet is kept constant and the amount of triacylglycerol is systematically increased is illustrated by the set of curves in Figure 1. B. The plasma LDL-cholesterol concentration increases as the amount of triacylglycerol containing predominantly saturated fatty acids is progressively increased in the diet. In contrast, there is a small but detectable decrease in the plasma LDL-cholesterol concentration when the triacylglycerol consists predominately of unsaturated fatty acids (4).

Thus, it is clear from these types of experimental results, which were obtained in a variety of animals, including humans, that there must be complex interactions between these three major types of dietary lipids: cholesterol, saturated fatty acids, and unsaturated fatty acids.

Metabolism of plasma LDL cholesterol

In the steady state, the concentration of LDL cholesterol is determined by the rate of entry of LDL into the plasma space

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relative to the rate of its removal from the circulation (Fig 2) (6, 7). The rate of LDL-cholesterol production is largely determined by the rate at which very-low-density lipoproteins are metabolized to LDL (8). LDL cholesterol is removed from the plasma by two different transport processes. One process is mediated by the LDL receptor (9, 10) and, therefore, is saturable. The second process is receptor independent (11) and its rate is a linear function of the plasma LDL-cholesterol concentration (12). Of the LDL that is transported from the plasma each day, ≈70–75% is removed by the receptor-dependent process in animals on a low-cholesterol, low-triacylglycerol diet (11, 13). This value is ≈58% in humans consuming a typical Western diet (14). Of the receptor-dependent transport that can be detected in the whole animal, ≥75% takes place in the liver (14, 15). As triacylglycerol and cholesterol are added to the diets of such animals, however, there is progressive suppression of receptor activity, and the steady-state plasma LDL-cholesterol concentration increases (3, 15).

The rate at which LDL cholesterol is taken up by a particular organ or by all of the tissues of the body (Jω) is equal to the sum of the rates of uptake by the receptor-dependent (Jr) and receptor-independent (Ji) transport processes in that organ or whole animal. Because Ji equals (J"Cω)/[(Kω + Cω)] and Jω equals PCω, it follows that Jr for any organ or for the whole animal can be calculated from the following expression (12):

\[ Jω = Jr + Ji = (J"Cω) / [(Kω + Cω)] + PCω / (Kω + Cω) \]  

where J"ω is the maximal receptor-dependent transport velocity (a reflection of receptor number), Kω is the plasma concentration of LDL cholesterol at which one-half of this maximal transport rate is achieved (a reflection of receptor affinity), Cω is the concentration of plasma LDL cholesterol in a given animal, and P is the proportionality constant for LDL-cholesterol transport by way of the receptor-independent pathway. This equation can be rearranged to give an expression that defines how the plasma LDL-cholesterol concentration will change in the whole animal given any alteration in receptor number or LDL-cholesterol production (15) because, in the steady state, the rate of LDL-cholesterol removal from the plasma space must equal the rate of entry into the plasma.

**Dietary influences on LDL-cholesterol concentration**

Although there are four variables in the above equation, studies have demonstrated that dietary lipids affect the plasma LDL-cholesterol concentration primarily by changing the amount of receptor activity in the whole animal and the rate of LDL-cholesterol production (6, 7). The theoretical relationship between changes in these two variables and the steady-state plasma LDL-cholesterol concentration are illustrated by the curves shown in Figure 3. The two solid curves in each panel show how the plasma LDL-cholesterol concentration will increase as receptor activity is progressively decreased to zero, when the LDL-cholesterol production rate is either 100% or 200% of the control value.

These two theoretical curves (15), which can be generated for most species, illustrate the interaction of receptor activity and production rate in dictating steady-state plasma LDL-cholesterol concentrations. When the production rate is kept constant at 100%, loss of receptor activity does not dramatically increase the plasma LDL-cholesterol concentration until > 50% of receptor activity has been lost. The amount of receptor activity, however, does markedly influence the extent to which a change in LDL-cholesterol production alters the circulating LDL-cholesterol concentration. For example, doubling LDL-cholesterol production in the presence of 100% receptor activity increases the LDL-cholesterol concentration by only ≈0.78 mmol/L (30 mg/dL). In contrast, if receptor activity is only 25%, doubling the LDL-cholesterol production rate increases the plasma LDL-cholesterol concentration by nearly 3.23 mmol/L (125 mg/dL).

The data points superimposed on these theoretical curves show actual experimental results when cholesterol or various triacyl-

![Figure 1](image1.png)

*FIG 1. Plasma LDL-cholesterol concentrations in hamsters fed increasing amounts of cholesterol with and without added triacylglycerol (TAG) (A) and in hamsters fed increasing amounts of triacylglycerol with a constant amount of cholesterol (B). All animals were fed for 30 d. T ± SE. From references 3 and 4.*

![Figure 2](image2.png)

*FIG 2. Three major processes that determine the plasma LDL-cholesterol (LDL-C) concentration. In the steady state, the rate of LDL-cholesterol production must equal the rate at which LDL cholesterol is removed from the plasma by both the receptor-dependent and receptor-independent processes. The receptor-dependent process is saturable whereas the rate of receptor-independent LDL-cholesterol uptake is a linear function of the plasma LDL-cholesterol concentration.*
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Figure 3. Plasma LDL-cholesterol concentrations plotted as a function of LDL-receptor activity and the LDL-cholesterol production rate in animals fed variable amounts of cholesterol and triacylglycerol (TAG) for 30 d. A: animals fed increasing amounts of cholesterol and no added triacylglycerol. B: animals fed no triacylglycerol, 20% hydrogenated coconut oil (HCO), or 20% safflower oil (SO) with no added cholesterol. C: animals fed each of the diets in B with 0.12% added cholesterol. From references 3 and 4. The theoretical curves were generated by using experimental values appropriate for male hamsters (12, 15). The LDL-receptor activity and LDL-cholesterol production rates were set at 100% for animals fed no added triacylglycerol or cholesterol. Superimposed on these theoretical curves are experimental data obtained after feeding the diets.

Glycerols are fed to such experimental animals, in this case the hamster. For example, Figure 3, A, illustrates the changes in these two variables when experimental animals are fed increasing amounts of cholesterol. When the new steady state is reached, there is a dose-dependent reduction in receptor activity coupled with a small increase in the LDL-cholesterol production rate. As a result of these two changes, the steady-state plasma LDL-cholesterol concentration increases from \( \approx 0.65 \) mmol/L (25 mg/dL) in the control animals to nearly 2.59 mmol/L (100 mg/dL) in the animals receiving 0.24% cholesterol in the diet (3).

Figure 3, B, shows the effects of feeding triacylglycerols alone to such experimental animals. With no added dietary cholesterol, such triacylglycerols have relatively little effect on LDL-receptor activity, the LDL-cholesterol production rate, or the steady-state plasma LDL-cholesterol concentration (4). Thus, as already illustrated in Figure 1, A, triacylglycerols containing either saturated or unsaturated fatty acids have little effect on LDL-cholesterol metabolism when the diet contains little or no added cholesterol. However, when a constant amount of cholesterol is added to such diets, then, as shown in Figure 3, C, adding triacylglycerol containing predominantly saturated fatty acids [hydrogenated coconut oil (HCO)] causes further loss of receptor activity and a significant further increase in the LDL-cholesterol production rate. As a consequence of these two changes, these saturated fatty acids increased the plasma LDL-cholesterol concentration to \( \approx 4.91 \) mmol/L (190 mg/dL). In contrast, when triacylglycerols containing predominantly unsaturated fatty acids [safflower oil (SO)] are added to these diets, there is partial restoration of receptor activity and a small decrease in the plasma LDL-cholesterol concentration below that seen with cholesterol feeding alone (4). Thus, saturated fatty acids fed with cholesterol cause a further suppression of receptor activity and a significant increase in the LDL-cholesterol production rate. Unsaturated fatty acids, however, tend to restore receptor activity. These changes, in turn, account for the marked increase in plasma LDL-cholesterol concentrations seen after feeding saturated lipids and for the small decrease in plasma LDL-cholesterol concentration found after feeding unsaturated fatty acids.

Chemical changes in the liver with lipid feeding

The studies illustrated in Figures 1 and 3 illustrate the well-known effects of feeding cholesterol and triacylglycerols containing predominately saturated or unsaturated fatty acids to a variety of animal species, including humans. Triacylglycerols fed with cholesterol can profoundly alter hepatic LDL-receptor activity and the LDL-cholesterol production rate and so change the steady-state LDL-cholesterol concentration. Presumably, these effects are articulated at the cellular level as the liver cell becomes enriched with both dietary cholesterol and dietary fatty acids. It is well known, for example, that the steady-state concentration of cholesteryl esters in liver cells increases in direct proportion to the amount of cholesterol in the diet (16, 17). Presumably, when net sterol delivery to the liver is progressively increased, both the putative regulatory and ester pools of sterol in the liver increase in parallel. This relationship is almost certainly dictated by the enzyme acyl CoA:cholesterol acyltransferase (ACAT). In this situation the steady-state concentration of cholesteryl esters in liver cells is directly proportional to the amount of cholesterol absorbed across the gastrointestinal tract (17), whereas hepatic receptor activity is inversely proportional to the amount of sterol reaching the liver (3). Consequently, hepatic receptor activity varies inversely with the cholesteryl ester concentration in liver cells.

The liver also becomes enriched with the fatty acids derived from dietary triacylglycerols. This is best seen in experiments in which single fatty acids are fed until new steady states are achieved. Such experiments are illustrated in Figure 4. In Figure 4, A, for example, the animals were fed triacylglycerols containing the 14:0 saturated fatty acid (18). The liver became relatively enriched with the 14:0 fatty acid and, in addition, the 16:0 fatty acid, whereas there was a relative decrease in the 9-cis 18:1 fatty acid. Similarly, when the 16:0 (Fig 4, B) or 18:0 (Fig 4, C) fatty acid was fed, there was relative enrichment with the respective...
dietary fatty acid and, in both cases, a relative decrease of the 9-cis 18:1 compound. When the two 18:1 isomers were fed (Fig 4, D and E), each became relatively enriched in the liver.

Such findings have raised the possibility that dietary triacylglycerols might also affect the amount of ACAT activity in the liver. Not only does this enzyme respond to cholesterol feeding, but the esterification reaction is very sensitive to the types of fatty acids available in liver cells. The preferred substrate for the esterification reaction is the 9-cis 18:1 fatty acid (20). The acyl-CoA derivatives of the other fatty acids may actually inhibit the esterification reaction (20, 21). Thus, these findings raise the possibility that enrichment of the liver with specific fatty acids could either inhibit or drive the esterification reaction so that the pool of cholesterol that is the putative regulator of hepatic receptor activity is profoundly altered. Fatty acids such as the 9-cis 18:1 compound, for example, might drive cholesterol into the ester pool, reduce the size of the putative regulatory pool, and enhance hepatic receptor activity. In contrast, other fatty acids that either inhibit the esterification reaction or displace the cellular 9-cis 18:1 fatty acid might block the esterification reaction, expand the putative regulatory pool of sterol, and further suppress hepatic receptor activity.

Effects of specific fatty acids on plasma LDL-cholesterol concentrations

To test these possibilities, experimental animals were fed diets that contained a constant amount of cholesterol and triacylglycerols containing a single fatty acid. When new steady states were achieved, LDL-cholesterol metabolism variables and plasma LDL-cholesterol concentrations were measured. In all of the studies, hamsters were used as the experimental animal because of the similarity of their cholesterol metabolism to that of humans.

The data shown in Figure 5 illustrate the effects of feeding the series of saturated fatty acids from 6:0 to 18:0 (18). The shorter-chain-length fatty acids—6:0, 8:0, and 10:0—did not significantly elevate plasma LDL-cholesterol concentration (Fig 5, A) and did not alter either LDL-receptor activity (Fig 5, B) or the LDL-cholesterol production rate (Fig 5, C) compared with the control animals that received only dietary cholesterol. These fatty acids are rapidly oxidized in the liver to acetyl CoA and so do not alter the fatty acid composition of the various lipid pools within the liver. In contrast, the liver became enriched with the longer-chain-length fatty acids when these were fed for a prolonged time. Under these circumstances the 12:0, 14:0, and 16:0 fatty acids significantly suppressed hepatic receptor activity (Fig 5, B), increased LDL-cholesterol production (Fig 5, C), and thus nearly doubled the steady-state plasma LDL-cholesterol concentration (Fig 5, A). However, even though the 18:0 fatty acid became enriched in the liver, it did not significantly alter either LDL-receptor activity or production rate and thus did not change the plasma LDL-cholesterol concentration. Similar results were found in human studies (22–24). Thus, of the saturated fatty acids commonly encountered in animal and human diets, only the 12:0, 14:0, and 16:0 compounds appear to alter LDL-cholesterol.

FIG 5. Effect of saturated fatty acid chain length on plasma LDL-cholesterol concentrations (A), relative hepatic LDL-receptor activity (B), and relative LDL-cholesterol production rate (C). *, Hamsters fed 10% saturated fatty acids that varied in chain length from 6 to 18 carbon atoms, 10% olive oil, and 0.12% cholesterol. ○ Control animals fed diets containing cholesterol and olive oil. The control value was set at 100% in panels B and C. \( \bar{x} \pm SE \). * Significantly different from the control values, \( P < 0.05 \). From reference 18.
terol metabolism. The 18:0 fatty acid, in contrast, apparently is biologically neutral with respect to LDL metabolism.

**Figure 6** shows similar experiments in which the metabolic effects of the C-18, monounsaturated fatty acids were examined (19). When the liver is enriched with the 9-cis 18:1 fatty acid, the preferred substrate for the ACAT reaction, there was a significant increase in hepatic receptor activity (Fig 6, B) and a reduction in the LDL-cholesterol production rate (Fig 6, C) so that the steady-state LDL-cholesterol concentration dropped to nearly half the control value (Fig 6, A). In contrast, the 9-trans 18:1 fatty acid, which cannot be utilized by ACAT for cholesterol esterification (20), did not change the amount of hepatic receptor activity nor the plasma LDL-cholesterol concentration. Studies in humans have also shown that consumption of the 9-cis 18:1 fatty acid causes a lower plasma LDL-cholesterol concentration than does the 9-trans 18:1 compound (25–27). Thus, the 9-trans 18:1 isomer, like the 18:0 compound, is biologically neutral with respect to regulation of hepatic LDL-receptor activity.

**Relationship of steady-state hepatic receptor activity and cholesteryl ester concentration**

There are fundamental differences in the relationship between steady-state hepatic cholesteryl ester concentrations and hepatic LDL-receptor activity when the experimental animal is fed either cholesterol or triacylglycerol. These differences are illustrated by the examples shown in Figure 7. In groups of animals fed different amounts of dietary cholesterol until a new steady state is achieved, hepatic LDL-receptor activity is inversely related to the concentration of cholesteryl esters in the hepatocyte. In contrast, when dietary cholesterol is kept constant at 0.12% and the animals are fed either the 9-cis 18:1 fatty acid or the 14:0 compound, the concentration of cholesteryl esters is directly related to LDL-receptor activity in the liver.

Although these differences may appear contradictory, these findings are consistent with the view that ACAT is critical in determining the size of the putative regulatory pool of sterol in the liver and, further, that ACAT activity is driven by the availability of both cholesterol and fatty acids within cells. When increasing amounts of cholesterol are added to the diet, there is presumably a parallel expansion in the steady-state concentration of both the putative regulatory pool of cholesterol and the amount of cholesterol stored as cholesteryl esters. Hence, in this situation hepatic receptor activity varies inversely with the cholesteryl ester concentration in cells. In contrast, when the amount of cholesterol reaching the cell is kept constant, enrichment of the liver with the 9-cis 18:1 fatty acid shifts the equilibrium of the ACAT reaction towards the ester pool whereas enriching the liver with the 14:0 compound suppresses this reaction. Hence, when the variable is the type of fatty acid available in liver cells, steady-state cholesteryl ester concentrations are directly related to receptor activity. This model is also consistent with the observation that the magnitude of the effect of dietary triacylglycerol on hepatic receptor activity (Figs 1 and 3) is dependent on the amount of cholesterol also present in the diet.

**Summary of the normal mechanisms of regulation of LDL-cholesterol concentrations by dietary lipids**

These various findings emphasize the interaction between dietary cholesterol and triacylglycerol in regulating steady-state concentrations of LDL cholesterol. When the diet contains only cholesterol, there is a nearly linear relationship between the steady-state concentration of hepatic cholesteryl esters and the amount of cholesterol in the diet. Hepatic receptor activity varies
inversely with these concentrations of cholesteryl esters. Under these circumstances there are modest increases in the concentration of LDL cholesterol that are directly related to the amount of cholesterol in the diet.

The addition of triacylglycerol to the diet may shift the equilibrium relationship between the cholesterol in the putative regulatory pool and the sterol in the inert cholesteryl ester pool. Fatty acids that are the preferred substrate for ACAT shift this equilibrium into the cholesteryl ester pool and increase hepatic receptor activity. Fatty acids that actually inhibit ACAT presumably expand the putative regulatory pool of cholesterol in the cell and suppress hepatic receptor activity.

Although the 9-cis 18:1 fatty acid is very active in altering hepatic LDL-receptor activity, the two isomeric forms of this fatty acid [the 18:0 and 9-trans 18:1 compounds] do not appear to regulate hepatic LDL-receptor activity. Enriching the liver with either of these compounds does not change the amount of receptor-dependent LDL-cholesterol transport nor the LDL-cholesterol production rate, which can be attributed to the content of cholesterol in the experimental diets. Such compounds, like the shorter-chain-length saturated fatty acids, appear to be biologically neutral, therefore, with respect to regulating hepatic LDL-receptor activity and steady-state plasma LDL-cholesterol concentrations.

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References